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Variations of Time-to-Incapacitation and Carboxyhemoglobin Values in Rats Exposed to Two Carbon Monoxide Concentrations

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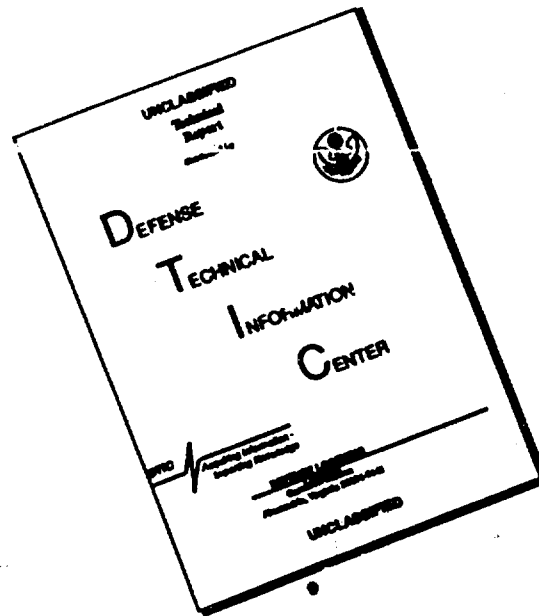DTIC
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The use of animals for this study was approved by the Institutional Laboratory Animal Use Review Committee under the U. S. Department of Agriculture Animal Welfare Act. The animals were cared for and humanely used in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals, PHS Publication (NIH) No. 86-23, revised 1985.

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16. Abstract

It has been suggested that passenger protective breathing equipment protect aircraft passengers from smoke for 5 min during an evacuation phase and for 35 min during an in-flight-plus-evacuation phase. Carbon monoxide (CO) is one of the most abundant smoke components and is the major threat in most fire atmospheres. Time-to-incapacitation (t_i) is an end-point related to escape from a fire, and variations in t_i measurements at specific CO concentrations have not been statistically documented. Therefore, variance in t_i at two CO concentrations that produce 5- and 35-min t_i in rats were determined; blood carboxyhemoglobin (COHb) saturation at t_i was measured. Fifty male Sprague-Dawley rats were individually exposed to each CO concentration in a chamber equipped with a rotating cage, and t_i was recorded as the time from insertion of the animal into the cage until it could no longer walk. In another set of experiments, blood COHb saturation was determined in rats exposed to these CO concentrations at stepwise intervals less than t_i. At incapacitation and at the end of each exposure interval, rats were quickly removed from the cage and killed for blood collection and COHb quantitation. Values (mean ± SD) for measured parameters were: For the 5-min t_i study, CO = 5706 ± 178 ppm, t_i = 5.0 ± 0.36 min, and COHb = 80.8 ± 1.3%; for the 35-min t_i study, CO = 1902 ± 33 ppm, t_i = 34.8 ± 6.8 min, and COHb = 71.2 ± 1.0%. COHb levels tended to plateau prior to incapacitation. Coefficients of variation for t_i were 7.2% and 19.5% for the 5- and 35-min study, respectively. The significant difference in the two COHb levels and the approach of COHb to a maximal level before incapacitation suggest that blood COHb saturation levels may not necessarily be indicative of the onset of incapacitation.

17. Key Words Inhalation toxicology; combustion gas/ carbon monoxide; time-to-incapacitation; carboxyhemoglobin; carbon monoxide uptake; animal exposure system	18. Distribution Statement Document is available to the public through the National Technical Information Service, Springfield, VA 22161

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VARIATIONS OF TIME-TO-INCAPACITATION AND CARBOXYHEMOGLOBIN VALUES IN RATS EXPOSED TO TWO CARBON MONOXIDE CONCENTRATIONS

INTRODUCTION

Inhalation of carbon monoxide (CO) is recognized as a primary cause of deaths in aircraft fire accidents. Viable escape options during in-flight fires do not exist for 20 to 30 min before the aircraft can land (Crane, 1984), and survival scenarios usually span a shorter period of time in postcrash fires. Both government and industry have initiated the development of passenger protective breathing equipment (PPBE) to increase survivability in fires (McFadden, et al., 1967; Higgins, 1987). Specific performance criteria for the evaluation of such devices have only recently been defined by the European Organization for Civil Aviation Equipment (EUROCAE, 1991); toxic gases and their concentrations generally present in aircraft fires and PPBE maximum pass limits that would provide the wearer a sufficient time to escape from a fire were considered. In general, it is viewed that PPBE at least protect individuals for 5 min during evacuation and for 25 min (20 min + 5 min) during in-flight-plus-evacuation. However, an in-flight period of 30 min may be necessary in certain fire scenarios.

An individual incapacitated from combustion gases has a minimal chance to escape from a fire, and onset of incapacitation may vary among subjects. Therefore, variations in the onset of this response at specific fixed concentrations of toxic combustion gases for the evacuation periods should be evaluated. Concentrations of combustion gases in blood also need to be quantitated to correlate with the response. CO is generated in potentially lethal amounts during fires and can produce incapacitation (Crane, et al., 1989; Hartzell, 1989; Gad, 1990). Time-to-incapacitation (t_i) is an applicable endpoint for predicting escape time from a fire environment (Crane, et al., 1977; Spurgeon, et al., 1979; Sanders, et al., 1991), and blood COHb saturation levels are routinely measured in CO poisonings to establish the severity of CO exposure. Relationship of the COHb levels with CO exposure concentrations and exposure times is not totally defined, but models do exist for CO uptake in non-human primates and humans (Peterson and Stewart, 1972; Kaplan and Hartzell, 1984).

This study was designed to determine variations in t_i among the laboratory rats exposed to the CO concentrations that would incapacitate the subjects at 5 and 35 min, representing durations of fire exposure in typical aircraft accidents; blood COHb saturation levels were determined at incapacitation. Uptakes of CO at the 2 concentrations, measured as increases in the COHb levels as a function of exposure time, were included to establish whether CO exposure concentration, t_i, and COHb parameters are interrelated. Information from this study may be useful in interpreting postmortem COHb levels in fire victims.

MATERIALS AND METHODS

Animals
Male rats of Sprague-Dawley origin were obtained from the Charles River Breeding Laboratories, Wilmington, MA, in a 100 to 125 g weight range. They were inspected by a veterinarian upon arrival and held in isolation for 8 days prior to use. Animals were allowed food and water ad libitum during, and following, the isolation period. All animals were fasted overnight before the CO exposure experiments. Rats (4 to 6 per cage) were housed in stainless steel cages (61 cm L X 45 cm W X 27 cm H) with mesh-wire floors in the Institute's centralized animal care facility maintained at 22 to 24°C with a relative humidity of 40 to 60% and a 12-hr on/off fluorescent light cycle (lights on 7 a.m. to 7 p.m.).

Animal Exposure Chamber
The animal exposure chamber used in the study was constructed from 1.3-cm polymethylmethacrylate sheets; its internal dimensions were: 50.8 cm L X 26.6 cm W X

Figure 1. Animal Exposure Chamber.

1. CO-air inlet; 2. Mixing fan assembly (1/15 hp motor, 5,000 rpm, fitted 7-cm, 4-bladed Nylon fan); 3. Ventilation holes, 12-mm diameter, cut through center divider of rotating cage; 4. Exposure chamber walls constructed from 1.3-cm polymethylmethacrylate; 5. Rotating cage assembly (divider and outer rim are 6-mm polymethylmethacrylate; surface is polyethylene mesh); 6. Ports sealed with rubber septa; 7. Access-door for animal insertion and removal; 8. Thumbscrew fasteners; 9. Polyethylene mesh cover, mesh openings are approximately 7-mm square; 10. Center divider and support for rotating cage; 11. Cage drive motor (4-rpm); 12. Cross supports for chamber rims and plastic mesh cover.
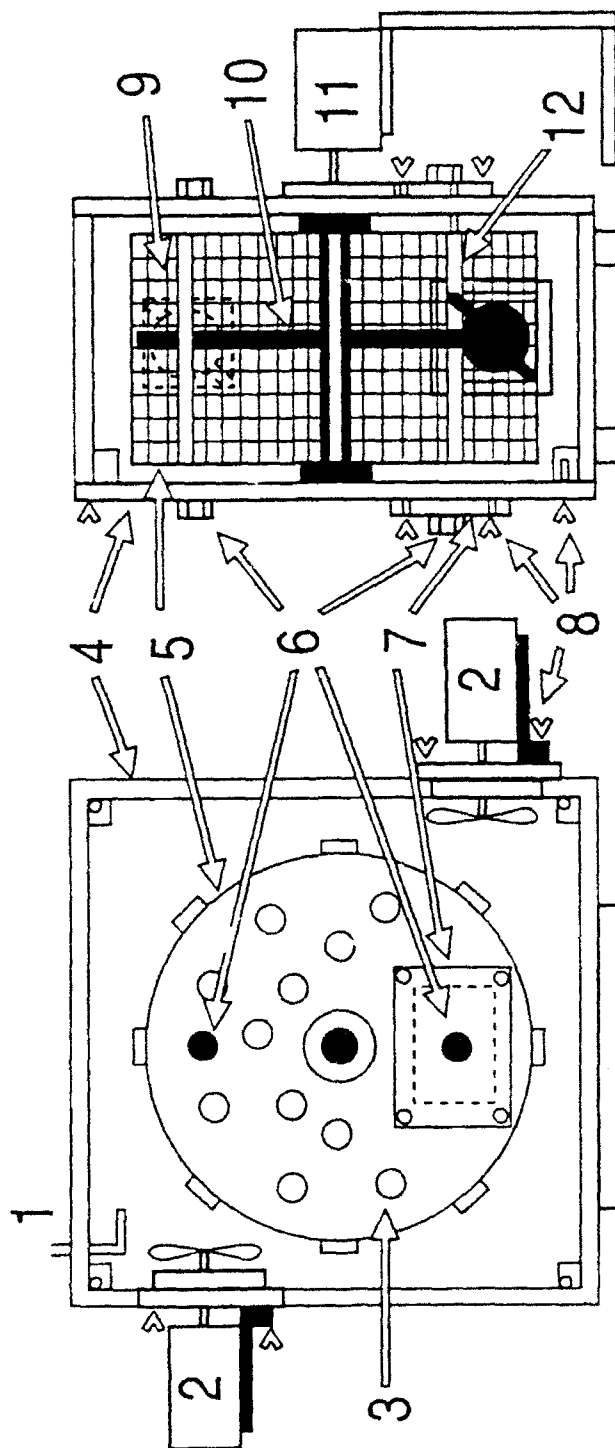
50.6 cm H (Figure 1). In the chamber, there was a cylindrical cage (40.5 cm diameter; 25.0 cm W) divided vertically into 2 equal compartments, each 12.5-cm wide and rotated horizontally by a 4-rpm geared motor to provide a circumferential velocity of 8 5 cm/sec. The front compartment of the cage was used for the animal tests. A gasketed access-door (10.4 cm X 10.4 cm) on the front panel of the chamber at the cage floor level allowed rapid animal insertion into, and removal from, the cage. There were 2 fans, 1 on each side of the chamber; 1 fan was at the upper part and the other at the lower part of the chamber. These fans were for homogeneous mixing and circulation of the gas-air mixture in the flow-through chamber atmosphere. Also, there were 2 ports sealed with rubber septa on the front side of the chamber.

Compressed CO (99.5%) and breathing air, purchased locally in cylinders, were mixed by passing through a baffled cylindrical mixing tube before entering the animal chamber. CO and air flow rates were regulated automatically using Scott model 5850E m iss flow controllers attached to a Scott model 5878A power supply/control unit (Scott Environmental Technology, Inc., Plumsteadville, PA). The gas-air mixture input was through a port in the top of the chamber, and an exhaust port was located on the rear panel. The entire chamber was installed in a fume hood into which the chamber exhaust was vented.

Experimental Protocol

Preliminary CO concentrations for producing incapacitation at 5- and 35-min exposure times were determined from the concentration-t_i curve defined in a previous study (Crane, et al., 1989). To refine the CO concentrations, 37 rats were exposed to CO over a range from 4658 to 6151 ppm that would result in incapacitation at 5 min and 19 rats to concentrations from 1298 to 2249 ppm to produce incapacitation at 35 min. Concentrations produced by flow rates of (25 mL CO + 4 L air)/min and (8.5 mL CO + 4 L air)/min were selected for the 5- and 35-min t_i study, respectively; these flows correspond to theoretical CO concentrations of 6211 and 2120 ppm.

The dynamic gas-air mixture flows through the chamber prevented changes in CO concentrations during animal insertion, exposure, or removal. Initial tests indicated that ambient O_2 levels were maintained for single rat exposures by the nominal 4-L/min flow of CO-air mixtures through the chamber. Consequently, O_2 was not monitored during subsequent animal exposure experiments.

Prior to exposure experiments, a volume of pure CO slightly less than that required to bring the chamber atmosphere to the desired concentration was injected into the closed chamber. With fans and rotating cage activated, the CO-air mixture was allowed to flow through the chamber, while sampling, until the stable CO concentration corresponding to the desired level was reached. The fans and cage motor were turned off, sampling timer was set to zero, and retaining screws in the chamber access-door were removed. In rapid sequence, the door was opened, a rat was inserted, the door was closed, and the timer, cage motor, and fans were activated.

The t_i was recorded as the time from insertion of the rat until it could no longer walk (i.e., when it began to tumble or slide) in the rotating cage, as subjectively determined by 2 individuals. Fifty rats were individually exposed to CO at each of the 2 flow rates to determine variations of t_i and of COHb at incapacitation. Additional rats were exposed at each flow rate and removed at intervals (3 rats/interval) less than t_i to determine the rate of increase in blood COHb saturation at each CO level; exposure intervals were 1, 2, 3, and 4 min for the 5-min experiments, while they were 2.5, 5, 10, 15, and 25 min for the 35-min tests.

COHb Determination

At incapacitation or at the end of each exposure interval, rats were removed from the chamber and killed by cervical dislocation. The body cavity was quickly opened, and blood was drawn into a 2.5-cc heparinized syringe from the vena cava at the point where it enters the right atrium. Without air contact, blood was immediately aspirated from the syringe by the sampling probe of a model IL 282 CO-Oximeter (Instrumentation Laboratories, Inc., Lexington, MA). The instrument was modified for use with rat blood; it determines COHb by measuring the absorbance of diluted blood samples at 4 wavelengths in the visible spectrum. Reagents and chemicals used in the analysis were obtained from the CO-Oximeter manufacturer. Each sample was analyzed in

Table 1. Time-to-Incapacitation (t_i) and Carboxyhemoglobin (COHb) Values for Rats Exposed to Two Carbon Monoxide (CO) Concentrations.

Parameters	Values[a, b]		
	Mean (Range)	SD	CV%
For 5-Min Study			
CO (ppm)	5706 (5402 - 6001)	178	3.1
t_i (min)	5.0 (4.2 - 5.8)	0.36	7.2
COHb (%) at t_i	80.8 (78.1 - 83.9)	1.3	1.6
For 35-Min Study			
CO (ppm)	1902 (1787 - 1945)	33	1.7
t_i (min)	34.8 (23.5 - 53.0)	6.8	19.5
COHb (%) at t_i	71.2 (69.4 - 73.7)	1.0	1.4

[a]Mean values are derived from rats individually exposed to CO; n = 50 for CO and t_i values, and n = 49 for COHb values. SD = Standard Deviation; CV = Coefficient of Variation, (SD + Mean) x 100.

[b]The corresponding data from which values were calculated are given in Tables 1 and 2 of the Appendix.

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triplicate; results were expressed as an average percent of blood COHb saturation for the 3 replicate analyses.

Chamber CO Concentration Measurement

Prior to and during the exposure, the chamber CO concentration was monitored by a Carle series 100 gas chromatograph (GC) equipped with 3.2-mm (i.d.) packed columns and a thermistor detector. A continuous flow of chamber atmosphere (55 mL/min) was pumped from the port on the access-door by a ceramic-piston pump (FMI model RRP, Fluid Metering, Inc., Oyster Bay, NY), through the GC sample loop, then back to the chamber. Sample injections onto to the GC were controlled by activation of an injector valve by an interval timer. Besides the zero-min injection, injections were at 1 min after rat insertion and subsequently at 1.5-min intervals. CO concentrations in samples were determined from standard curves, which were prepared daily from syringe dilutions of tank CO in air.

The concentration-exposure time curves were essentially straight horizontal lines from the time zero to the end of exposure. This observation suggested that (i) any CO concentration changes during the animal exposures were minimal and (ii) the gas concentration at incapacitation (or the end of the applicable selected exposure intervals) was almost identical to that in the chamber sample immediately preceding incapacitation (or the exposure interval). Therefore, CO concentrations at incapacitation (or the end of the exposure intervals) were estimated by extrapolating the values of the preceding concentrations to the t_i or exposure time. The exposure concentration of CO for each experiment was calculated by the integration of chamber CO concentration as a function of exposure time from $t = 0$ to $t = t_i$ (or exposure time) and dividing the resulting product by t_i (or exposure time), i.e.,

$$Exposure\ Concentration = \frac{\int_{t=0}^{t=t_i} Cdt}{t_i}, \quad (1)$$

where C = CO concentration in ppm and t = exposure time in min.

Statistics

The normality of distribution of measurements was determined by performing the Kolmogorov-Smirnov one-sample test at $\alpha = 0.05$ (Miller and Miller, 1988; Wilkinson, 1989). Where possible, data were analyzed at $\alpha = 0.05$ using the analysis of variance and Tukey's HSD multiple comparison test for statistical pairwise differences between the groups (Wilkinson, 1989). Otherwise, the significance of differences between means was checked by the Student's t-test (SigmaPlot, 1991). A difference between means was considered significant at $p \leq 0.05$. Values are presented as the mean ± SD.

RESULTS

As given in Table 1, 5706 ± 178 and 1902 ± 33 ppm CO were effective in producing 5- and 35-min t_i, respectively; coefficients of variation for these CO concentrations were correspondingly 3.1 and 1.7%. The distribution of t_i values at 5706 ppm CO was uniform, showing a spread of 1.6 min from minimum to maximum with the 5.0-min mean; the low 1902 ppm CO produced a much wider t_i value range of 29.5 min with the 34.8-min mean. Blood COHb saturation levels at t_i were 80.8% for 5706 ppm CO and 71.2% for 1902 ppm CO ($p \leq 0.05$). Although these COHb levels were significantly different from each other, their coefficients of variation were essentially identical, i.e., 1.6% for the high CO and 1.4% for the low CO. Except for the COHb values, the CO concentration and t_i measurements for the 5- and 35-min experiments had normal distributions (Figure 2); these measurements were not statistically different from their corresponding standard normal population forms ($p > 0.05$).

Mean CO exposure concentrations for the 5- and 35-min uptake study were 5680 ± 77 (n = 12) and 1846 ± 36 (n = 15) ppm, respectively. Within each set of studies, the CO concentrations for the exposure intervals were not statistically different from each other (p > 0.05). As exhibited in Figure 3, the blood COHb saturation increased progressively with duration of CO exposure, as well as with CO concentration. At 5680 ppm CO, the COHb saturation increased rapidly, with a tendency to reach an equilibrium level by the 5-min exposure time.

Figure 2. Distribution of CO Exposure Concentrations, t_i Values, and COHb Levels for the 5- and 35-Min Study.

The frequency distributions were based on the measurements derived from 50 rat experiments for CO concentration and t_i and from 49 rat experiments for COHb for each of the 2 studies. The normality of distribution of measurements was established by the Kolmogorov-Smirnov one-sample test at $\alpha = 0.05$. Details are given in the text.
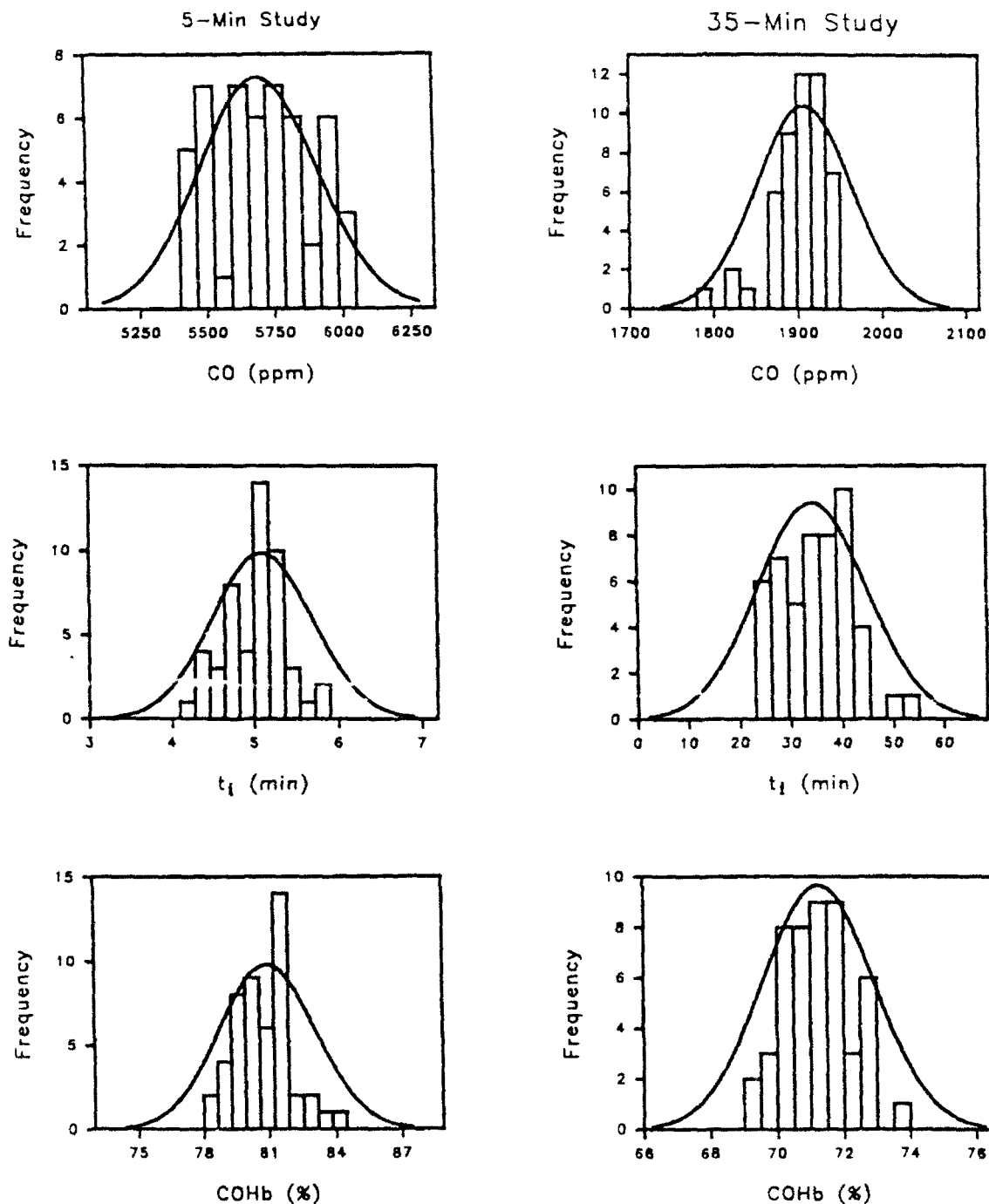
Figure 3. COHb Levels as a Function of Exposure Time at Two CO Concentrations.

Rats were individually exposed to 5680 ± 77 (n = 12) ppm CO for 1, 2, 3, and 4 min and to 1846 ± 36 (n = 15) ppm CO for 2.5, 5, 10, 15, and 25-min periods (3 rats/interval). The CO exposure concentrations were calculated from t = 0 to t = "exposure interval" using Equation (1) given in the text and represent the mean of all exposure intervals within each set of the uptake studies. CO concentrations for each exposure interval, including t_i, did not significantly change from each other within 5- or 35-min study (p > 0.05). At the end of the exposure period, each rat was removed from the chamber and killed; blood was collected and analyzed for COHb. Details are given in text. Each point represents the mean of the COHb determinations from 3 rats; bars depict SD for each mean value. The points marked "t_i" at 5 and 35 min represent the mean of COHb values for 3 animals incapacitated at exactly 5 and 35 min, respectively, during the t-variation study. The data from which these values were calculated are listed in the Appendix (Tables 3 and 4).
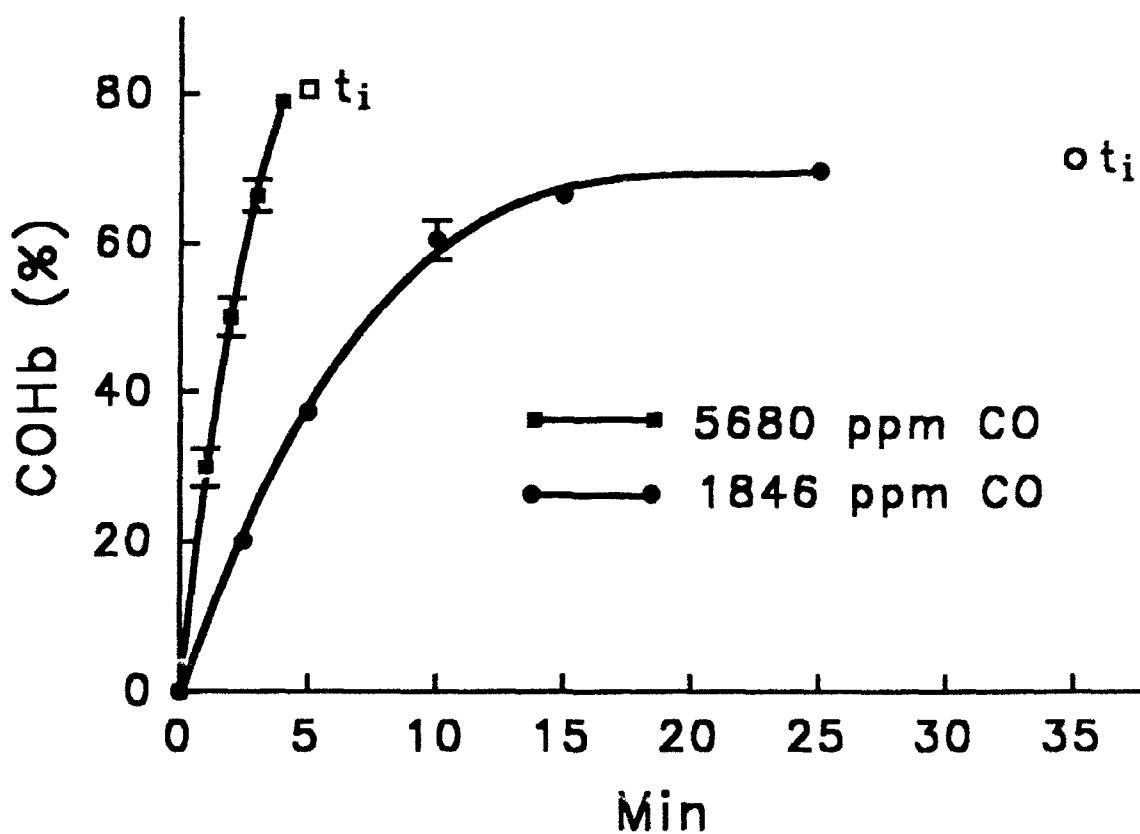
The COHb saturation increase was also observed at 1846 ppm CO, but the increase was at a slower rate than that noted at the high CO concentration; the COHb saturation started reaching the steady state by approximately 15 min. For the high CO concentration, the COHb values at the 4-min exposure time and at the 5-min t were not different from each other ($p > 0.05$), but these COHb values were different from 1-, 2-, or 3-min exposure time COHb value. Similar comparisons of COHb data points from the low CO exposure showed no difference between the 15- and 25-min COHb value or between the 25-min and 35-min t COHb value; however, the difference between the COHb levels at the 15- and 35-min time point was significant ($p \leq 0.05$).

DISCUSSION

The CO exposure concentrations of 5706 and 1902 ppm were determined to produce nominal 5- and 35-min t, respectively. The modest variations in the CO concentrations suggested that the fluctuations observed in t values were primarily associated with the individual animal response. However, the variations in the 35-min t values, at least in part, could have been because of the increasing difficulty in precisely judging this end-point, where the response onset is not sharp at the low CO concentration. This variability is supported by the limited validity of Haber's rule that the CO exposure concentration (C: ppm) and exposure time (t; min)

product within certain gas concentration and exposure time ranges is a constant (Packham and Hartzell, 1981). At low gas concentrations, the rule loses applicability and variations in the incapacitation response become more pronounced (Crane, et al., 1989). The accumulated C•t dose of 28530 ppm•min for 5706 ppm CO and the 5-min t was in reasonable agreement with the doses reported for incapacitation in humans (35000-45000 ppm•min), juvenile baboons (34250 ppm•min), and active cynomolgus monkeys (21000-30000 ppm•min) (Kaplan and Hartzell, 1984), and in rats (30000-40000) (Kaplan and Hartzell, 1984; Kaplan, 1988; Gad, 1990). The higher C•t value of 66190 ppm•min for the 35-min t might be a reflection of the inapplicability of Haber's rule and could be associated with the tolerability of lower CO concentrations by the animals, but this value was considerably lower than the C•t values that cause lethality in the rats (Kaplan, 1988; Gad, 1990). The COHb level was significantly higher at the high CO concentration (low C•t value) than the low CO concentration (high C•t value).

Although the COHb levels at incapacitation for the 2 CO concentrations were not constant, the COHb values were in agreement with the values from the equation developed by Hartzell and coworkers (1989) to predict the %COHb saturation at incapacitation in rats (Table 2). The blood COHb saturation levels of > 65% at incapacitation and of > 75% at death in rats have been

Table 2. Comparison of Experimental Blood Carboxyhemoglobin (COHb) Saturation Values with Predicted Values from the Hartzell Equation[a].

CO (ppm)	%COHb Blood Saturation at Incapacitation	
	Found (n = 49)	Predicted
1902	71.2	70.2
5706	80.8	82.1

[a]Hartzell, et al (1989).

documented by Kaplan (1988). Sokal (1975) has indicated that rats showed only faint reaction to mechanical stimuli at a COHb level of 50-51% after exposure to CO in a dynamic-flow metabolic chamber. Purser and Berrill (1983) found that sedentary monkeys reached 40% COHb levels before clear indications of incapacitation were noted, but that active monkeys were severely intoxicated at 33% COHb levels; it was also indicated in the study that a sedentary human with a COHb level of 30% might become rapidly incapacitated upon initiation of sudden physical activity in a fire situation. Gettler and Freimuth (1940) have questioned whether postmortem COHb findings could define if a person was alive or dead when first coming in contact with a fire and cited fatal CO poisonings with 30-40% COHb. The average blood COHb saturation levels of 40% (Mayes, 1991) and 45% (Veronneau, et al., 1992) have been reported in victims of 2 aircraft fire accidents. The possible contribution of other combustion products (e.g., hydrogen cyanide) in causing fatalities, however, cannot be ruled out in individuals succumbing to smoke exposures.

The COHb levels in the uptake study were nearly identical to those predicted by an equation developed by Hartzell, et al. (1989) for modeling COHb loadings *versus* CO concentrations. The patterns of CO uptake by the blood were clearly consistent with the computer-constructed curves from their equation, showing that COHb reaches a steady state that is proportional to the CO exposure concentration. At 1902 ppm CO, postmortem COHb analysis for any animal killed after 15 min would have indicated an incapacitating (or lethal) level of COHb, when, in fact, the animal was neither incapacitated nor dead. The very small changes in COHb between the time the "plateau" (steady-state) level is reached and incapacitation occurs, and the significant difference between the 5- and 35-min t_i COHb level, require that we consider the recurrent question: "Do blood COHb levels correlate with the degree of CO intoxication?" and its corollary, "Can postmortem COHb levels reliably indicate antemortem incapacitation?" Goldbaum (1976) has shown that dogs bled to an anemic state and, then, infused with CO-saturated erythrocytes to obtain a COHb saturation of about 60% showed no symptoms of CO toxicity, but other dogs breathing CO died with COHb saturations averaging

65%; it was concluded that the presence of elevated concentrations of COHb does not cause symptoms of toxicity and that the more probable mechanism was that dissolved CO, in plasma, competes at the cellular level with O_2 for cytochrome a_3. The blood COHb level is indeed an indication of severity of CO exposure, but may not be directly linked to the onset of incapacitation. Other parameters, such as plasma CO, could be directly related to incapacitation. Therefore, the onset of incapacitation might be more associated with the plasma CO concentration than the blood COHb saturation. Whatever the actual mechanism, incapacitation, in our study, was defined as the simple loss of ability to continue walking in a motor-driven, rotating cage, where enforced physical activity was continuous from insertion to incapacitation; the more complex issues of visual and judgmental impairment were not addressed. However, the observation that the animals were capable of apparently normal physical activity for a considerable time after 66-70% COHb levels were reached is significant.

SUMMARY AND CONCLUSIONS

The CO exposure concentrations that produced incapacitation at 5 and 35 min were correspondingly 5706 and 1902 ppm; coefficients of variation in these CO concentrations were 3.1 and 1.7%, respectively. Fifty laboratory rats exposed to the high CO concentration showed an overall response (t_i) variation of 7.2%, and an additional 50 rats exposed to the low CO concentration responded with an overall variation of 19.5%. Blood COHb saturation levels at incapacitation varied with the CO concentration to which the animal was exposed, reaching 80.8% saturation at 5706 ppm and only 71.2% at 1902 ppm; these COHb levels were significantly different from each other ($p \leq 0.05$).

No specific COHb level could be linked to the onset of incapacitation, as the blood COHb level reached a plateau before incapacitation occurred at both CO concentrations. For example, at the lower CO concentration, the COHb level reached an equilibrium level by 15 min that remained essentially unchanged, when the animals were incapacitated at approximately 35 min. Thus, it would appear that postmortem COHb concentrations may not be reliable indicators of antemortem

incapacitation from CO inhalation and that appropriate caveats should be applied when making judgments on the basis of COHb analyses only.

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APPENDIX

TIME-TO-INCAPACITATION (t_i) VALUES AND CARBOXYHEMOGLOBIN (COHb) LEVELS AT INCAPACITATION FOR RATS EXPOSED TO CARBON MONOXIDE (CO)

Table 1. Data for 5-Min Study.

Rat No.	t_i (min)	CO* (ppm)	COHb (%)	Rat No.	t_i (min)	CO* (ppm)	COHb (%)
1	5.1	5664	79.4	26	4.5	5730	78.1
2	4.7	5644	78.4	27	5.0	5704	79.6
3	5.2	5777	79.9	28	5.5	5723	80.7
4	5.2	5744	81.3	29	5.0	5733	80.9
5	5.3	5753	81.4	30	5.7	5459	83.9
6	5.3	5770	80.6	31	5.1	5506	81.4
7	4.6	5750	79.2	32	4.3	5479	79.5
8	4.4	5951	78.9	33	5.1	5472	81.3
9	4.8	5879	79.6	34	4.2	5485	80.2
10	5.2	5993	80.2	35	4.8	5498	79.9
11	4.9	5991	82.3	36	5.8	5604	82.6
12	5.2	5942	80.9	37	5.4	5610	82.5
13	5.0	5950	81.6	38	5.1	5645	81.5
14	5.0	5499	81.4	39	4.6	5705	81.7
15	4.8	5539	80.2	40	4.8	5835	78.7
16	5.0	5627	80.7	41	5.3	5421	81.6
17	4.8	5609	80.0	42	5.3	5423	81.2
18	4.4	5688	--	43	5.1	5402	81.5
19	4.9	5880	80.6	44	4.3	5419	79.8
20	5.1	5925	80.6	45	5.2	5842	81.9
21	5.5	6001	83.6	46	4.8	5842	79.7
22	5.1	5952	80.7	47	5.3	5823	81.9
23	4.9	5951	81.7	48	5.8	5791	83.2
24	4.9	5657	78.8	49	5.0	5817	81.7
25	5.1	5712	80.1	50	4.8	5490	80.4

*CO exposure concentration, see text for definition. Insufficient blood was obtained from rat no. 18 to analyze for COHb.

Table 2. Data for 35-Min Study.

Rat No.	t_i (min)	CO* (ppm)	COHb (%)	Rat No.	t_i (min)	CO* (ppm)	COHb (%)
1	25.0	1902	70.6	26	23.5	1899	69.7
2	40.2	1908	70.8	27	28.5	1893	70.1
3	28.5	1874	71.8	28	27.0	1896	70.2
4	41.0	1879	71.5	29	35.5	1905	70.6
5	53.0	1882	72.6	30	24.5	1926	70.2
6	23.8	1866	70.1	31	30.2	1924	70.2
7	36.5	1919	71.9	32	37.5	1926	72.7
8	25.5	1910	69.6	33	27.5	1938	71.4
9	30.0	1916	70.3	34	39.7	1929	73.7
10	37.0	1917	71.3	35	30.2	1933	--
11	28.0	1900	69.4	36	40.8	1939	72.0
12	36.2	1787	70.4	37	27.0	1939	70.1
13	49.0	1826	71.8	38	37.8	1936	72.3
14	32.0	1823	71.0	39	39.5	1937	72.7
15	27.0	1889	70.6	40	33.7	1937	71.6
16	33.0	1890	71.4	41	36.8	1926	71.0
17	39.5	1845	72.4	42	34.0	1911	71.8
18	43.0	1880	71.3	43	45.0	1900	72.7
19	39.5	1876	71.7	44	39.5	1907	71.1
20	40.0	1885	72.6	45	31.5	1901	69.5
21	35.0	1908	71.0	46	34.0	1930	70.8
22	34.0	1887	71.4	47	38.0	1933	72.7
23	44.5	1889	71.4	48	42.5	1932	72.2
24	40.0	1885	71.2	49	25.5	1931	69.7
25	36.5	1901	71.6	50	34.0	1945	71.8

*CO exposure concentration, see text for definition. Insufficient blood was obtained from rat no. 35 to analyze for COHb.

Table 3. Data for Exposure Periods Less than t,
at Nominal 5680 ppm CO.

Rat No.	Exposure Time (min)	CO (ppm)	COHb (%)
1	1.0	5621	30.5
2	1.0	5646	32.0
3	1.0	5706	27.0
4	2.0	5821	47.1
5	2.0	5719	51.7
6	2.0	5745	51.5
7	3.0	5541	67.0
8	3.0	5719	68.1
9	3.0	5686	64.0
10	4.0	5567	80.5
11	4.0	5704	77.9
12	4.0	5684	78.5
13	5.0*	5725	81.5
14	5.0*	5627	80.7
15	5.0*	5704	79.6

*Data for rats listed at the 5-min exposure time were selected from the animals in the Appendix Table 1 for the purpose of comparison only; these animals (No. 13, 14, and 15) were incapacitated at the time of blood removal for COHb analyses.

Table 4. Data for Exposure Periods Less than t₁
at Nominal 1846 ppm CO.

Rat No.	Exposure Time (min)	CO (ppm)	COHb (%)
1	2.5	1797	20.3
2	2.5	1826	20.4
3	2.5	1860	19.8
4	5.0	1891	38.2
5	5.0	1816	36.7
6	5.0	1836	37.1
7	10.0	1888	59.4
8	10.0	1884	58.6
9	10.0	1852	63.5
10	15.0	1881	66.1
11	15.0	1787	66.0
12	15.0	1845	67.4
13	25.0	1792	70.6
14	25.0	1886	70.5
15	25.0	1852	67.9
16	35.0*	1908	71.0
17	35.0*	1930	70.9
18	35.0*	1945	71.9

*Data for rats listed at the 35-min exposure time were selected from the animals in the Appendix Table 2 for the purpose of comparison only; these animals (No. 16, 17, and 18) were incapacitated at the time of blood removal for COHb analyses.